SUGARCANE

Near-Infrared Reflectance Spectroscopy Analysis of Phosphorus in Sugarcane Leaves

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ABSTRACT

Rapid screening for high leaf P concentration may help sugarcane (interspecific hybrids of *Saccharum* spp.) growers in the Everglades Agricultural Area reduce P in discharge water, an important component of Everglades restoration. The purpose of this study was to evaluate near-infrared reflectance spectroscopy (NIRS) as a potential tool to analyze sugarcane leaf P concentration. Local calibrations for samples with similar spectral characteristics were calculated using modified partial least-squares regression for the following categories: parents, offspring, fertilizer rate, and water table. Additionally, global calibrations were calculated for 11 groupings of these local categories. Analyses for much of the study found that the most accurate local calibration was that of fertilizer rate, with $R^2 = 0.90$ and ratio of standard deviation (s) to standard error of cross validation = 2.17. However, further multiplicative scatter correction of spectral data and the elimination of unneeded wavelength segment points by Martens Uncertainty regression with software that became available later in the study resulted in nearly perfect prediction equations, with $r^2 = 0.99$ and ratio of s to standard error of prediction ≥ 32.0 for the offspring local equation and the parents + fertilizer rate + water table global equation. These results show that researchers not obtaining calibrations at desired levels of accuracy with NIRS should try to eliminate unneeded wavelength segments. Use of NIRS is proposed as a tool to provide rapid, accurate measurements of sugarcane leaf P content for characterizing commercial cultivars and for screening for high-P cultivars in breeding programs.

SUGARCANE IS THE PRIMARY CROP of the Everglades Agricultural Area (EAA) in South Florida, grown on approximately 134 000 ha of Histosols (Glaz, 2000). Phosphorus deficiency of sugarcane in the EAA may result in reduced cane tonnage, particularly in ratoon crops (Gascho and Kidder, 1979; Glaz et al., 2000). Contrarily, excessive P increases cane tonnage, with a correlated decrease in sugar concentration, or simply decreases sugar concentration (Gascho and Kidder, 1979; Glaz and Ulloa, 1994; Glaz et al., 2000). Excessive P application also may contribute to P enrichment of surface water (Coale et al., 1994). Most virgin Florida Histosols contain from 50 to 1360 mg P kg^{-1} (Chen and Ma, 2001), with a majority of the P tied up in organic compounds. Differences in P uptake among sugarcane cultivars could lead to the annual removal of 8.5 kg P ha^{-1} from EAA soils (Glaz et al., 1997). It would there-fore be helpful to develop sugarcane cultivars that produce high yields with less P fertilizer or cultivars that take up more soil P. Sugarcane in the EAA obtains 30 to 85% of its total P from soil P that was originally in the organic form but is mineralized to a plant-available form during the sugarcane growth cycle (Anderson, 1990). Under well-drained conditions, cultivated Histosols mineralize 6 to 87 kg P ha^{-1} yr^{-1} (Diaz et al., 1993). Phosphorus fertilizer rates are recommended to optimize sugarcane yields according to foliar analysis using wet chemistry digestion, coupled with proper interpretation of soil testing (Elwali and Gascho, 1984; Anderson, 1990; Glaz et al., 1997). Using traditional chemical digestion and analytical procedures for plant P concentration, however, is time consuming and expensive (Malley et al., 2000; Gillon et al., 1999). Perhaps foliar diagnosis of leaf P would be more widely used if results were more rapidly available so that corrective measures could be taken before P deficiencies caused yield losses.

Near-infrared reflectance spectroscopy is a rapid analytical method for measuring the chemical composition of materials and requires only simple sample preparation. Covalent bonds between light atoms such as C, N, H, O, and P generally absorb energy in the infrared region where they have fundamental vibrational frequencies and combination overtones detectable in the near-infrared region (400–2500 nm) (Malley et al., 2000; Gillon et al., 1999).

Calibration of a near-infrared spectrometer is an inferential process of deriving models that relate spectral readings of samples to values determined by reference chemistry. Calibration involves the selection of representative samples, acquiring spectra and reference analyses, data preparation, and statistical modeling and validation. To minimize interference from the spectra of strongly overlapping constituents and from light scatter variations, spectral data from many different wavelengths are combined by various methods, including multiple stepwise linear regression, partial least squares, and principal-component analysis (De Boever et al., 1994; Givens and Deaville, 1999).

Two broad categories of calibration equations have been used by researchers: local and global equations. Local equations are models developed for sample populations expected to have similar spectral characteristics, whereas global equations are progressively more univer-

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sal models developed from sample populations of broader spectral diversities (Gillon et al., 1999). Both local and global strategies have been used to predict N, P, and other minerals in a variety of plants (Clark et al., 1987; Vazquez de Aldana et al., 1995; Foley et al., 1998; Gillon et al., 1999; Ruano-Ramos et al., 1999). Abrams et al. (1987) and Shenk and Westerhaus (1993) concluded that broad-based equations are potentially as accurate as more local equations and offer the advantage of reducing the rather large effort required to assemble a calibration set and perform conventional wet chemistry analyses to develop calibration equations. Gillon et al. (1999) found that the accuracy of global calibrations is not systematically better than local calibrations though all of the global calibrations increased in robustness as they were valid for a wider range of concentrations of a constituent for spectrally heterogeneous materials. The successful estimation of mineral elements by NIRS may depend on the occurrence of these elements in either organic or hydrated molecules (Clark et al., 1987; Vazquez de Aldana et al., 1995).

Phosphorus is important in the formation of nucleic acids, phytate, and phospholipids (De Boever et al., 1994). However, the wavelengths chosen for P calibration of plant tissue using NIRS were not similar to those at which NIRS responses were noted when scanning phytate, potassium phosphate, or calcium phosphate (Clark et al., 1987). Calibrations obtained for total P in plant tissue were considered acceptable by Shenk et al. (1981), Clark et al. (1987), Gillon et al. (1999), and Ruano-Ramos et al. (1999) but needed further development in studies reported by Shenk and Westerhaus (1993) and Vazquez de Aldana et al. (1995). Although expandable equations for P concentrations in a variety of forage, barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), oat (*Avena sativa* L.), rye (*Secale cereale* L.), silage, sorghum [*Sorghum bicolor* (L.) Moench], and wheat (*Triticum aestivum* L.) samples have been documented by researchers as well as by NIRS instrument companies (Clark et al., 1987; Batten, 1998), there are no reports of NIRS calibration equations to predict P in sugarcane leaves. However, Meyer (1998) reported varying degrees of success for predicting N and Si in sugarcane leaves and other sugarcane traits such as photosynthesis rate, cane yield, and sugar content.

The purpose of this study was to evaluate NIRS as a potential tool for analysis of sugarcane leaf P concentration. Such information hopefully will help in developing successful foliar diagnosis, which could be useful for clonal screening in sugarcane breeding programs as well as for P recommendation in sugarcane production.

MATERIALS AND METHODS

Field Experiments

The relationship between reference chemistry and NIRS prediction of sugarcane leaf P concentration was determined with leaf samples from three field experiments with a wide range of genetic material and agronomic conditions for sugarcane in Florida. One experiment was designed to estimate the heritability of leaf P concentration in sugarcane. This experi-

ment was planted near Canal Point, FL, on 4 June 1999 on a Torry muck soil (Euic, hyperthermic Typic Haplosaprist). No P fertilizer was added to this experiment. Leaf samples were collected on 8, 20, and 27 Sept. 1999 and 17 Nov. 1999. Leaf samples from this and all other experiments were collected between 0900 h and 1500 h from the leaf immediately below the top visible dewlap and contained the midrib. This experiment contained plots of clonally propagated genotypes that were used in biparental crosses to generate genetically unique progeny planted from seeds. There were four male parents and five female parents, resulting in 20 crosses. The parents were planted in four replications, at the rate of 10 stools per replication. The offspring were also planted in four replications; each plot of offspring had up to 25 genetically unique plants from a biparental cross. Initially, two NIRS local calibration categories were developed from this experiment, parents and offspring. Chemical and NIRS analyses were conducted on 125 randomly selected samples of parents and 318 randomly selected samples of offspring (Table 1).

Offspring was later divided into two categories. One category was offspring for which one chemical analysis (one subsample) was conducted from the plant material analyzed in each scanned sample. The second category was offspring for which four chemical analyses (four subsamples) were conducted per scanned sample. There were 262 samples in the first offspring category and 56 in the second (Table 1).

A second experiment was performed to characterize the leaf P concentration of eight clonally propagated, high-yielding genotypes. An additional factor in this experiment was P fertilizer rate: 0, 24, and 48 kg P ha^{-1}. This experiment was planted on 22 Nov. 1995 near South Bay, FL, about 6 km north of the southernmost area where sugarcane is produced in Palm Beach County, on a Dania muck soil (Euic, hyperthermic, shallow Lithic Haplosaprist). Samples were collected on 9 July 1996 and 19 Aug. 1996. Local calibrations from this experiment were classified as fertilizer rate. Chemical and NIRS analyses were conducted on 98 randomly selected fertilizer rate samples (Table 1).

The local calibration for water table was derived from two experiments, which were conducted to determine the response of several high-yielding genotypes to three summer water tables. One experiment was planted 6 Feb. 1997 and the second on 19 Jan. 1999 on a Pahokee muck soil (Euic, hyperthermic Lithic Haplosaprist) about 12 km southeast of South Bay, FL. Phosphorus fertilizer was applied to all plots in all experiments at the rate of 13 kg P ha^{-1}. Samples were collected from the first-ratoon growth from the first water table experiment on 20 May 1998 and from the plant cane growth from the second water table experiment on 8 Oct. 1999. The first experiment contained nine and the second experiment 12 clonally propagated genotypes grown on three target summer water tables of 15, 38, and 61 cm below the soil surface. Chemical and NIRS

Table 1. Sample number (*n*), range, mean, and standard deviation (*s*) of reference chemistry values for five populations used for near-infrared spectroscopy calibration development for sugarcane leaf P concentration.

Calibration	*n*	Range	Mean	*s*	*s*/Mean
		——— g P kg^{-1} ———			
Parents	125	0.81–1.99	1.35b†	0.24	0.18
Offspring one subsample	262	0.12–2.17	1.34b	0.32	0.24
Offspring four subsamples	56	0.96–1.97	1.38b	0.22	0.16
Fertilizer rate	98	1.18–3.45	2.09a	0.51	0.24
Water table	142	0.55–1.88	1.41b	0.21	0.15

† Different letters in the same column indicate significant difference at α < 0.05 (*t* test).

Table 2. Calibration statistics of near-infrared reflectance spectroscopy with traditional chemistry analyses for sugarcane leaf P concentration using WinISI II software for five single categories (local equations) and 11 combinations of categories (global equations).

Category	n†	s‡	SEC§	SECV¶	R^2	s/SECV	Math#	Scatter††
			g P kg^{-1}					
Parents (PA)	66	0.25	0.16	0.18	0.59	1.39	0,4,4,1	SNV-D
Offspring (one subsample) (O1)	191	0.24	0.21	0.22	0.19	1.09	0,4,4,1	SNV-D
Offspring (four subsamples) (O)	44	0.23	0.18	0.22	0.37	1.05	2,4,4,1	None
Fertilizer rate (F)	71	0.52	0.17	0.24	0.90	2.17	2,4,4,1	SNV-D
Water table (W)	53	0.29	0.09	0.13	0.89	2.23	2,4,4,1	None
PA + O	102	0.23	0.16	0.19	0.36	1.21	1,4,4,1	SNV-D
PA + F	133	0.52	0.22	0.25	0.82	2.08	1,4,4,1	SNV-D
PA + W	116	0.22	0.18	0.19	0.38	1.16	0,4,4,1	SNV-D
O + F	111	0.54	0.21	0.27	0.85	2.00	2,4,4,1	SNV-D
O + W	93	0.22	0.19	0.19	0.28	1.16	0,4,4,1	None
F + W	118	0.50	0.17	0.20	0.89	2.50	1,4,4,1	SNV-D
PA + O + F	171	0.49	0.20	0.25	0.82	1.96	2,4,4,1	SNV-D
PA + O + W	155	0.23	0.19	0.19	0.30	1.21	1,8,8,1	SNV-D
PA + F + W	179	0.47	0.21	0.23	0.80	2.04	1,8,8,1	None
O + F + W	141	0.49	0.20	0.24	0.83	2.04	1,4,4,1	SNV-D
PA + O + F + W	216	0.44	0.22	0.24	0.78	1.83	1,4,4,1	None

† Number of samples used for calibration development selected by the computer software.
‡ s, standard deviation.
§ SEC, standard error of calibration.
¶ SECV, standard error of cross validation.
# The first number is the order of the derivative; the second is the segment length in data points over which the derivative was taken; and the third (first smooth) and fourth (second smooth) are the segment lengths over which the function was smoothed. The setting of 1 for the second smooth indicates that there was no second smooth.
†† SNV-D, standard normal variant and detrend; none, no scatter option.

analyses were conducted on 142 randomly selected water table samples (Table 1).

Spectrometry Measurements and Analyses

Sugarcane leaf samples were dried at 60°C, ground in a stainless-steel mill to pass a 1-mm screen, and then scanned with a NIRS instrument (Model 6500, Foss NIRSystems, Silver Spring, MD). Two replicated measurements of monochromatic light from a single sample cup were made at 2-nm intervals from 400 to 2500 nm to produce an average spectrum with 1050 data points. Reflectance (R) was converted to absorbance (A) using the following equation:

$$A = \log(1/R)$$

Calibrations were first conducted using the WinISI II (Foss NIRSystems, Silver Spring, MD) software package.

Local calibrations were produced from the following categories: parents, offspring (one subsample per NIRS scanned sample), offspring (four subsamples per NIRS scanned sample), fertilizer rate, and water table. Eleven progressively global calibrations were produced using various combinations of these categories (Table 2). The offspring data set with four subsamples per sample was used in all global equations developed with WinISI that included offspring. Principal-component analyses were performed on each data set before calibration. The WinISI II software was used to rank spectra in a file according to their standardized Mahalanobis distance from the average spectra of the file. If the standardized distance of a sample was >3.0, the sample was considered as an outlier and eliminated (Shenk and Westerhaus, 1991).

Reference Chemistry Analysis of Phosphorus

Chemical analyses were conducted on three subsamples for each scanned sample in the fertilizer rate and water table categories. Only one chemical analysis (one subsample) was done per scanned sample for the parent category and the offspring category with 262 samples (Table 1). Four chemical analyses (four subsamples) per scanned sample were conducted on samples in the second offspring set. Whenever more than one subsample was conducted, the mean of subsamples

for each sample was used for calibrations and predictions. For the offspring, parent, and water table experiments, 0.3 g of leaf tissue was digested using sulfuric acid and hydrogen peroxide (Lowther, 1980). For the fertilizer rate experiment, 0.1 g of leaf tissue was digested using nitric acid in microwave bombs (Amana Radarange, Amana, IA) at 70% power for 4 min and then at 100% power for 2 min (Rechcigl and Payne, 1990). After digestion, P concentration was determined by a modified molybdenum blue procedure (Murphy and Riley, 1962) at 880 nm using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instruments, Rochester, NY). Quality control samples were included to verify that there were no significant differences between the two digestion methods.

Near-Infrared Reflectance Spectroscopy Calibration of Phosphorus

Calibrations were developed for leaf P concentration (P_{leaf}) measured in grams per kilogram using a modified partial least-squares regression method (Shenk and Westerhaus, 1991). The following model was used:

$$P_{leaf} = b_0 + b_1A_1 + b_2A_2 + \ldots + b_nA_n$$

where $A_1, A_2, \ldots, A_n$ are n independent spectral variables, each with a combination of one or more spectral values (absorbance); $b_1, b_2, \ldots, b_n$ are n partial regression coefficients; and b_0 is the intercept.

Two different software programs were used to develop these chemometric models using all spectral and chemical data. The WinISI II software used modified partial least-squares regression to compare spectra and reference chemistry values and ultimately to predict NIRS P values using all wavelength segments between 400 and 2500 nm. Later, Unscrambler software (Version 7.6, CAMO, Trondheim, Norway) was used. The mathematical preprocessing used in the Unscrambler software was the multiplicative scatter correction with no derivatives on the log($1/R$) data. The multivariate method of partial least squares as described by Martens and Naes (1989) was used for predicting P concentration (dependent variable) from NIRS spectra with Unscrambler. The model was further refined by the use of Martens Uncertainty regression, in which those

wavelength segments (*x* variable) that were statistically not being helpful to the full-spectrum model were removed.

Cross validation was used to estimate the optimum number of samples in each calibration to avoid overfitting. This consisted of a form of Monte Carlo simulation, in which the population was arbitrarily divided into a small number of groups and a prediction was made of the values for one group based on calibrations developed from the remaining groups. In turn, overall predictions were made for groups with the mean of predictions for all groups (Shenk and Westerhaus, 1993). It is through this procedure that the WinISI II software calculated the standard error of cross validation (SECV) on independent samples.

During the calculations of cross validations, one of every four samples was randomly reserved from the ordered data sets. The algorithm was repeated four times, and all residuals of the four predictions were pooled to provide a SECV and R^2 on independent samples. The final model was then recalculated with all of the samples to obtain the standard error of calibration (SEC).

For each calibration, six mathematical treatments (0,4,4,1; 0,8,8,1; 1,4,4,1; 1,8,8,1; 2,4,4,1; and 2,8,8,1) combined with two scatter correction options, standard normal variant and detrend (SNV-D) or none, were compared across the entire spectrum (400–2500 nm) at 8-nm intervals. The first number of the mathematical treatment is the order of the derivative function; the second is the segment length in data points over which the derivative was taken; and the third (first smooth) and fourth (second smooth) are the segment lengths over which the function was smoothed. The setting of one for the second smooth indicates that there was no second smooth. The standard normal variant option scaled each spectrum to have a *s* of 1.0 and helped reduce particle size effects. A detrending procedure removed or reduced the linear and quadratic curvature of each spectrum. Calibration equations were judged for accuracy on the basis of their values of R^2 and *s*/SECV (Gillon et al., 1999).

Near-Infrared Reflectance Spectroscopy Prediction of Phosphorus

After choosing a calibration equation for each local or global category, the equation was tested for accuracy in predicting the sugarcane leaf P concentration of independent samples. This process included using part of the samples for calibration and the rest for prediction. In the current study, only four of every five samples were randomly selected for calibration, and the fifth sample was reserved for prediction. Shenk and Westerhaus (1993) reserved one in six, and Gillon et al. (1999) reserved one in four samples in similar studies.

Relationships between NIRS-predicted P (*y*) and reference chemistry P (*x*) from both WinISI II and Unscrambler are shown by simple linear regression:

$$y = a + bx$$

where *b* is the regression coefficient and *a* is the intercept. The ratio of standard error of prediction (SEP) to *s* of the prediction set (s_x), s_x/SEP, is RPD (Williams, 1987). The RPD and r^2 values were used to assess the suitability of prediction equations.

RESULTS AND DISCUSSION

Sugarcane Leaf Phosphorus Concentration by Reference Chemistry

Leaf samples from four populations of sugarcane were analyzed by reference chemistry (Table 1). Mean P concentration in sugarcane leaves from the parent, the offspring, and the water table categories were similar. Mean P concentration differed by about 1 *s* for the leaves sampled from the fertilizer rate category compared with the other categories. Cumulative frequency plots indicated that the parents and water table categories were from similar populations, whereas the offspring and fertilizer rate categories were not (data not shown). However, all mean values were within ranges of leaf P concentration previously reported for sugarcane in the EAA (Anderson, 1990; Glaz et al., 1997).

Near-Infrared Reflectance Spectroscopy Calibration of Phosphorus in Sugarcane Leaves with No Wavelength Segments Eliminated

Using principal-component analysis to measure the distance of each sample from the center of the spectral hypersphere, it was shown that samples from each category had similar spectral characteristics (data not shown). Four (0,4,4,1; 1,4,4,1; 1,8,8,1; and 2,4,4,1) of the six mathematical treatments in combination with two scatter options (SNV-D or none) resulted in the most accurate calibration for each local or global calibration (Table 2). Often with NIRS, as occurred in this study, optimum calibrations are obtained with various mathematical treatments. These mathematical treatments were similar to those of 1,8,8,1; 2,8,8,1; and 2,4,4,1 reported by Gillon et al. (1999) for P prediction in heterogeneous plant materials from different categories.

Calibration equations developed for P concentration in sugarcane leaves from different categories were characterized by their SEC, SECV, and R^2 (Table 2). Among global equations, R^2 ranged from 0.28 for the offspring + water table equation to 0.89 for the fertilizer rate + water table equation. The R^2 values of local equations ranged from 0.19 for the offspring equation calculated with one subsample to 0.90 for the fertilizer rate equation. The ratios of *s*/SECV ranged from 1.05 to 2.50 for all local and global categories. Six equations, two local (the fertilizer rate and the water table) and four global (the parents + fertilizer rate, the fertilizer rate + water table, the offspring + fertilizer rate, and the offspring + fertilizer rate + water table), had $R^2 \geq 0.82$ and *s*/SECV ≥ 2.0 (Table 2).

Nine equations had $R^2 \geq 0.78$ and SEC ≤ 0.22 (Table 2). These R^2 and SEC values were more accurate than those reported by Vazquez de Aldana et al. (1995) and Ruano-Ramos et al. (1999) for P in forage and grassland samples. Ruano-Ramos et al. (1999) concluded that their calibrations were useful for determining P concentrations of semiarid grasslands used for grazing regimes. The calibrations reported here were less accurate than those reported by Saiga et al. (1989) and Gillon et al. (1999) in heterogeneous plant materials.

Ruano-Ramos et al. (1999) and De Boever et al. (1994) reported specific wavelengths for estimation of P in grasses and vegetable feedstuffs. For other elements, a theoretical basis associating minerals and organic functional groups has led to the use of certain

Table 3. Prediction statistics for sugarcane leaf P concentration using equations developed from near-infrared reflectance spectroscopy (NIRS) calibrations using WinISI II software for five single categories (local equations) and 11 combinations of categories (global equations).

| Category | n† | Chemistry | | NIRS | | | | | |
		$\bar{x}$‡	s_x§	$\bar{y}$‡	s_y§	b¶	SEP#	RPD††	r^2
				g P kg^{-1}					
Parents (PA)	25	1.38	0.23	1.40	0.24	0.71	0.17	1.35	0.53
Offspring (one subsample) (O1)	53	1.32	0.36	1.38	0.11	0.81	0.35	1.03	0.06
Offspring (four subsamples) (O)	12	1.42	0.29	1.47	0.48	0.30	0.38	0.76	0.18
Fertilizer rate (F)	22	2.09	0.50	2.11	0.43	0.71	0.27	1.85	0.69
Water table (W)	29	1.27	0.19	1.22	0.13	0.70	0.17	1.12	0.22
PA + O	37	1.37	0.29	1.38	0.14	0.66	0.28	1.04	0.10
PA + F	48	1.59	0.46	1.68	0.47	0.78	0.31	1.48	0.63
PA + W	53	1.30	0.22	1.34	0.15	0.85	0.19	1.16	0.31
O + F	33	1.75	0.46	1.85	0.47	0.82	0.28	1.64	0.69
O + W	39	1.34	0.19	1.32	0.12	0.44	0.19	1.00	0.08
F + W	51	1.76	0.52	1.70	0.53	0.89	0.27	1.93	0.76
PA + O + F	46	1.62	0.46	1.72	0.48	0.82	0.28	1.64	0.71
PA + O + W	64	1.30	0.21	1.31	0.13	0.84	0.18	1.17	0.26
PA + F + W	75	1.49	0.42	1.44	0.39	0.91	0.24	1.75	0.71
O + F + W	62	1.61	0.54	1.61	0.44	1.03	0.29	1.86	0.72
PA + O + F + W	86	1.45	0.38	1.46	0.34	0.92	0.22	1.73	0.66

† Number of samples used for prediction.
‡ $\bar{x}$ and $\bar{y}$, arithmetic mean.
§ s_x and s_y, standard deviation.
¶ b, regression coefficient.
# SEP, standard error of prediction.
†† RPD, ratio of SEP to s_x.

wavelength ranges with NIRS. For example, Ca and Mg are associated with components of the cell wall. Calcium pectate may bind plant cells and has a reflectance spectrum in the near-infrared region (Vazquez de Aldana et al., 1995). The chlorophyll bands in the near-infrared region reported by Clark et al. (1987) were also close to the 2076 nm wavelength at which Vazquez de Aldana et al. (1995) had success calibrating Mg in grass samples. For our sugarcane samples, the equations developed by NIRS with the WinISI II software were more accurate when the entire spectrum from 400 to 2500 nm was used rather than when we selected wavelengths previously determined successful for P.

Most sugarcane research and commercial production is conducted with small numbers of clones. For example, 12 cultivars (each a clone) were grown on 80% of Florida's sugarcane production area in 2000 (Glaz, 2000). About 10 clones were used in each of the equations that calibrated P concentration for the parents, the fertilizer rate, and the water table. However, every plant used to generate the offspring equations was a genetically unique individual. The R^2 values were particularly low in the initial NIRS calibrations for P when using the offspring, whether generated by samples from one or four subsamples (Table 2).

Fertilizer rate was the most useful local calibration to use as a component in global equations before we removed unneeded wavelength segments. Combining spectral and chemical data from the fertilizer rate experiment with data from other experiments generally increased R^2 and s/SECV values for parents, offspring, and water table. This is possibly due to increased range, mean, and variation in sugarcane leaf P concentrations for fertilizer rate where mean = 2.09 g P kg^{-1} and s = 0.52 g P kg^{-1} (Table 1). Williams (1987) concluded that as the s increased, the R^2 could also be expected to

increase, provided there was satisfied precision of testing by chemical and NIRS methods.

Near-Infrared Reflectance Spectroscopy Prediction of Phosphorus in Sugarcane Leaves with No Wavelength Segments Eliminated

Calibration equations were used to predict leaf P concentration in samples that had not been used in the calibrations. Statistics were calculated to assess the accuracy of these predictions using the WinISI II software (Table 3). Acceptable predictions were characterized by low values of SEP and high values of r^2 and RPD. At least one global equation was found that included water table + fertilizer rate, with or without parents, or offspring that had $r^2 \geq 0.71$ and RPD ≥ 1.75.

Vazquez de Aldana et al. (1995) reported an r^2 of 0.53 and SEP of 0.31 g P kg^{-1} tissue for predicting P in grasses with NIRS. Similarly, Ruano-Ramos et al. (1999) reported r^2 values between 0.49 and 0.77 and SEP values in the range of 0.17 to 0.26 g P kg^{-1} tissue for predicting P concentration in grasses with NIRS. Compared to these previous studies, most of our SEP values were similar, and nine of our equations had similar r^2 values ($0.53 \leq r^2 \leq 0.75$). Malley et al. (2000) reported that for agricultural applications, it is desirable to have $r^2 > 0.95$ and RPD > 5.0. For screening, they suggested RPD > 2.5. Most NIRS work to analyze P in plant tissue has not yet met those standards. The most accurate local prediction equation using all wavelength segments in the present study was for the fertilizer rate, and the most accurate global equation was for the fertilizer rate + water table (Table 3 and Fig. 1 and 2). The lowest standards set by Malley et al. (2000) were not met in either of those prediction equations.
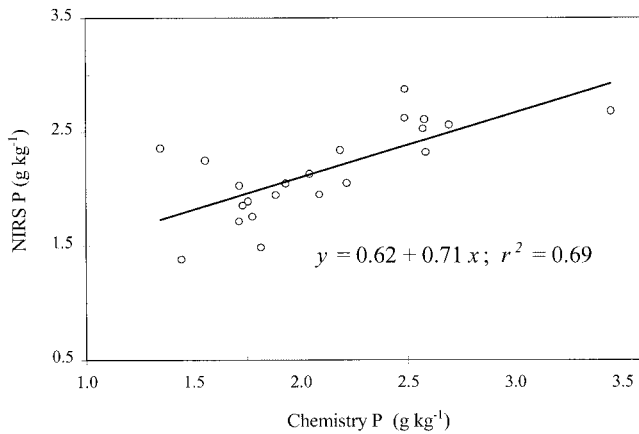
Fig. 1. Relationship between near-infrared reflectance spectroscopy (NIRS)–predicted P and reference chemistry P in sugarcane leaves from local fertilizer rate calibration using simple linear regression.

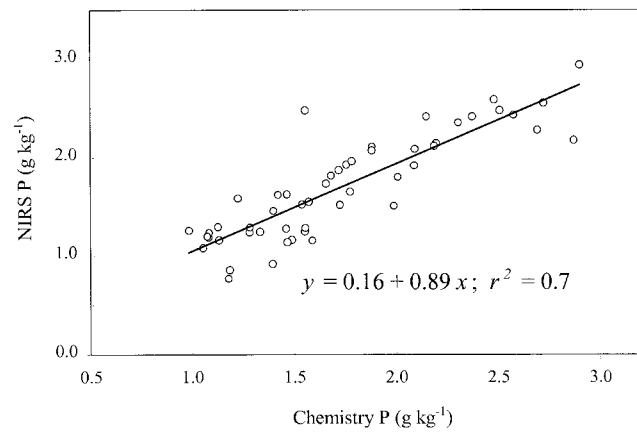$$y = 0.62 + 0.71\,x;\ r^2 = 0.69$$

Fig. 2. Relationship between near-infrared reflectance spectroscopy (NIRS)–predicted P and reference chemistry P in sugarcane leaves from global calibration of fertilizer rate and water table using simple linear regression.

$$y = 0.16 + 0.89\,x;\ r^2 = 0.7$$

Near-Infrared Reflectance Spectroscopy Calibration and Prediction of Phosphorus after Eliminating Unneeded Wavelength Segments

Martens Uncertainty regression was later used as an additional step for preprocessing of the spectral data before developing models by partial least-squares regression (Martens and Naes, 1989). By removing wavelength segments that were not helpful to their respective models, Martens Uncertainty regression, in combination with partial least-squares regression, generated nearly perfect predictions of leaf P between the reference chemistry analyses and NIRS for both categories of offspring (one and four subsamples) (Table 4). Earlier in the study, we were not making satisfactory progress with the offspring calibration, so we increased the number of reference chemistry subsamples per NIRS sample from one to four. However, the lack of improvement in the original calibration for offspring with four subsamples vs. one subsample (Table 2) compared with the near-perfect prediction equations developed by removing unneeded wavelength segments for both one and four subsamples (Table 4) suggests that the problem was not in reference chemistry variability.

The local calibrations and predictions of parents, fertilizer rate, and water table were not substantially improved by Martens Uncertainty (Tables 2, 3, and 4). In each of these three local categories, treatments consisted of about 10 vegetatively propagated clones. In the offspring category, each sample was of a genetically unique individual. The most robust global calibration included parents, fertilizer rate, and water table and resulted in RPD = 49.0 and $r^2 = 0.99$ (Table 4 and Fig. 3). Generally, adding offspring data to other calibration sets had neutral or negative effects. These results demonstrate that researchers using NIRS should develop separate calibrations for studies with hundreds of sugarcane progeny rather than plan to analyze these progeny with global calibrations that were developed from several clonally propagated genotypes.

Malley et al. (2000) set the criteria that for agricultural applications, it is desirable to have $r^2 > 0.95$ and RPD > 5.0. By removing unneeded wavelength segments from our original data, we surpassed these criteria for each category in either a local or global calibration. A prediction equation was developed for each category with $r^2 = 0.99$ and RPD ≥ 32.0. Martens Uncertainty regression was a powerful tool for model development of sugarcane leaf P with NIRS. The local and global approaches were

Table 4. Calibration and prediction statistics for sugarcane leaf P concentration from near-infrared spectroscopy using Martens Uncertainty for single categories (local calibrations) and selected combinations of categories (global calibrations).

Category	n†	s‡	SEC§	SECV¶	b#	SEP††	RPD‡‡	r^2
				g P kg^{-1}				
Parents (PA)	111	0.24	0.16	0.18	0.44	0.18	1.3	0.61
Offspring (one subsample) (O1)	242	0.32	0.01	0.01	0.99	0.01	32.0	0.99
Offspring (four subsamples) (O)	56	0.22	0.02	0.02	0.99	0.02	11.0	0.99
Fertilizer rate (F)	85	0.51	0.26	0.30	0.68	0.30	1.7	0.80
Water table (W)	125	0.21	0.16	0.17	0.34	0.17	1.2	0.55
PA + O	159	0.23	0.17	0.17	0.43	0.17	1.4	0.63
F + W	235	0.55	0.19	0.21	0.85	0.21	2.6	0.92
PA + F + W	371	0.49	0.06	0.01	0.99	0.01	49.0	0.99
PA + O1 + O + F + W	614	0.47	0.24	0.25	0.61	0.25	1.9	0.77

† n, number of samples. Unscrambler used all samples that contributed wavelengths segments, unlike WinISI, which did not use samples providing similar information.
‡ s, standard deviation of reference chemistry values.
§ SEC, standard error of calibration.
¶ SECV, standard error of cross validation.
\# b, regression coefficient.
†† SEP, standard error of prediction.
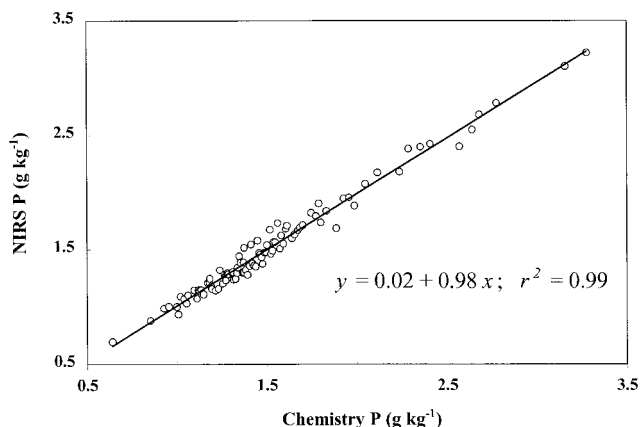‡‡ RPD, ratio of SEP to s.

Fig. 3. Relationship between near-infrared reflectance spectroscopy (NIRS)–predicted P and reference chemistry P in sugarcane leaves from global calibration for parents, fertilizer rate, and water table data calculated using Martens Uncertainty regression.

effective with the two broad categories of sugarcane data sets, offspring and clonal genotypes. The offspring was composed of many progeny, all different genetically, and the clonal genotypes were composed of relatively few (often about 10) clonally propagated genotypes. A robust local calibration was developed for the progeny, whereas pooling data from different experiments using clonally propagated genotypes resulted in a separate, robust calibration.

Near-infrared reflectance spectroscopy offers several advantages to traditional chemical procedures required for P analysis of plant tissue. Except for calibration, there is no need to use hazardous chemicals. This reduces the risk for accidents and the amounts of stored dangerous chemicals and hazardous waste. After calibrations have become robust, only small numbers of reference chemistry samples are needed with NIRS. Thus, a major advantage of NIRS is that it allows researchers to process greater numbers of samples per experiment or increase the number of experiments. Samples analyzed by NIRS would only need to be collected, dried, and ground. Once ground, an employee places the sample material in a sample cup and scans it on the NIRS instrument. The process takes from 3 to 5 min. per sample. In our laboratory, one employee was able to analyze about 10 samples by NIRS for every sample that a team of three employees could analyze by the chemistry procedures.

CONCLUSION

Robust calibrations for leaf P concentration were developed locally for offspring and globally for parents, water table, and fertilizer rate. These calibrations are essentially at the same level of accuracy as their reference chemistry values. The offspring calibration has an immediate application to screen thousands of progeny annually in a breeding program. The global calibration of clonally propagated material can be applied to quickly analyze P in the leaves of sugarcane growing in commercial fields. Rapid determination of leaf P allows for a precision-farming application that applies mini-

mum P to a crop while obtaining optimum yields, thereby minimizing environmental losses of P.

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